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Reproduction and Development of the Conspicuously Dimorphic Brittle Star *Ophiodaphne formata* (Ophiuroidea)

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Abstract. *Ophiodaphne formata* is a conspicuously dimorphic ophiuroid; the disk diameters are approximately 1 mm for males and 5 mm for females. The dwarf male clings to the larger female, with the oral surfaces and bursae of the paired ophiuroids closely appressed. Moreover, the female of each pair adheres aborally to the oral surface of a host sand dollar, *Astriclypeus manni.* Spawning and external fertilization occur in August, at Tsuruga Bay, Sea of Japan. Development of the dimorphic brittle star *O. formata* is described for the first time, from spawning through metamorphosis, with special attention to the formation of the skeletal system and the external morphology of early juveniles. Fertilized eggs are about 90 μm in diameter, pale pink, and negatively buoyant. The embryos undergo equal, total, and radial cleavage, and the larval skeleton first forms as a pair of tetraradiate spicules. Larval development proceeds to an 8-armed planktotrophic ophiopluteus, with skeletal elements that consist of a body rod and two recurrent rods. Three weeks after fertilization, all the pluteal arms, except for the postero-lateral arms, are absorbed, and the metamorphosing larvae sink to the bottom. Metamorphosis is completed 21.5 days after fertilization, and the resulting juvenile is pentagonal and approximately 270 μm in diameter. The smallest specimen (480 μm in disk diameter) collected by field sampling exhibited male features on the skeletal plates of the jaw and disk. Sexual dimorphism, the peculiar pairing behavior, and the close relationship with the host sand dollar may have evolved as distinct reproductive characteristics in this ophiuroid with its typical ophiopluteus larvae.

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Introduction

The biology of reproduction has been reported in various echinoderms, and they are mostly dioecious (Hyman, 1955; Delavault, 1966; Lawrence, 1987). Sexual dimorphism is not common, but some species show external morphological differences in size, genital papillae, genital pores, and arm spines (Hyman, 1955; Delavault, 1966; Tyler and Tyler, 1966; Lawrence, 1987; O'Loughlin, 2001; Stöhr, 2001). In a few ophiuroids—*Ophiosphaera insignis, Amphiura scripta,* and *Astrochlamys bruneus*—the difference in size between males and females is very large (Brock, 1888; Koehler, 1904; Mortensen, 1933, 1936). In *O. insignis* and *A. scripta,* a dwarf male pairs with a much larger female, clinched mouth to mouth; and in *A. bruneus,* a smaller male attaches to the dorsal surface of a larger female. However, no spawning has been observed in these ophiuroids. Therefore, the pairing of a male and a female in these dimorphic species has not been demonstrated as a distinct reproductive behavior. Ophiuroid reproduction and development has been reviewed by Hyman (1955), Strathmann and Rumrill (1987), Hendler (1995), and Byrne and Selvakumaraswamy (2002), but neither the larva nor the metamorphosis of a dimorphic species has been described.

We have been studying an unusual sexually dimorphic ophiuroid, *Ophiodaphne formata,* which has two novel characteristics. First, the dwarf male and the larger female are coupled mouth to mouth, and we have observed this pairing throughout the year, even in the nonbreeding season. Second, these paired ophiuroids are only found firmly attached to the oral surface of a host sand dollar, *Astriclypeus manni.* The ophiuroid *O. formata* ranges from the Arabian coast to Indonesia (Koehler, 1905; Guille, 1981) and was recorded from off Minabe, Wakayama Prefecture (Honshu), Japan, by Irimura (1981), who identified it, at first, as *Ophiodaphne materna.* He also reported that a large specimen and a smaller one—supposedly female and male, respectively—were found on the oral side of a sand dollar, *Clypeaster reticulatus,* clinched together mouth to mouth. However, the sex of the larger and smaller specimens was not verified in this very brief report. Later, Irimura and his coauthors (2001) classified specimens of this ophiuroid, which were collected at depths of 25.5–40 m, as *O. formata.*

In view of the unusual natural history of *O. formata,* the present study was initiated to confirm that pairing in this species is a reproductive behavior. Pairs of *O. formata* comprising a dwarf male and a much larger female were removed together from the oral side of the host sand dollar, *A. manni,* and kept in glass beakers. We observed spawning; then external fertilization occurred; and the fertilized eggs developed into 8-armed ophioplutei, which metamorphosed into juveniles. Thus, the developmental mode of the sexually dimorphic *O. formata* has now been defined.

Materials and Methods

In the summers of 1999 to 2001, adult sand dollars (*Astriclypeus manni*) were collected from depths of 5 m by scuba diving on the sandy bottom of Tsuruga Bay, Fukui Prefecture (central Japan, 35° 44′ N, 136° 03′ E). The sand dollars were examined for samples of paired and unpaired, young and adult *Ophiodaphne formata.* The ophiuroids (n = 245) were found on only about 1 out of 10 sand dollars, and 46% of them were paired (n = 112). When pairs were found, they were carefully removed from the sand dollars with fine forceps and placed into glass beakers containing filtered seawater. In 2002, about 20 individuals of *O. formata* were collected every 2 months for histological study of gonadal development.

A few days after the collection in August 2000, spawning occurred naturally, with no artificial stimuli, and the fertilized eggs were removed from the glass vessels and reared in 5-l glass beakers; density was maintained at one larva per 10 ml of filtered seawater. The water temperature was about 26 °C, approximately that at the collection site. Larval cultures were agitated with a plastic propeller rotating at 60 rpm. Seawater used for culture was obtained from the open sea and was filtered many times and renewed every 3 days. A small quantity (3 ml/l) of larval food in the form of a mixture of unicellular algae—*Dunaliella tertiolecta, Isochrysis galbana,* and *Chaetoceros gracilis*—was added to the culture when the seawater was changed.

The development of embryos and larvae, including skeletal formation, was observed by both light microscopy and polarized light microscopy. Measurements of living embryos and larvae were made with an ocular micrometer. For scanning electron microscopy, metamorphosing larvae and juveniles were fixed for 1 h in 2% OsO_4 in a 50 m*M* Na-cacodylate buffer (pH 7.4); the osmolarity of the fixative was adjusted by adding sucrose (final concentration 0.6 *M*), according to Komatsu *et al.* (1990). The fixed materials were dehydrated in an ethanol series, dried with a critical-point dryer (Hitachi, HCP-2), coated with gold-palladium (Hitachi, E101 Ion Sputter), and examined with a Hitachi S-2000 scanning electron microscope.

Histological observations of the reproductive organs were made to confirm their sex and maturity. Fresh specimens were measured, dissected, and fixed in Allen-Bouin's solution, followed by decalcification with 5% trichloroacetic acid for one week at 4 °C. These prepared gonadal tissues were serially sectioned at 4 μm by a routine paraffin method and stained with Delafield's hematoxylin and eosin.

Results

Pairing, symbiosis, and sexual dimorphism

Adult individuals of *Ophiodaphne formata* are diecious, and the disk diameter of mature specimens is about 1 mm in males and 5 mm in females. The oral surface of the dwarf male is pressed against the oral surface of the larger female, and the arms of the male cling to the female at the interradius position (Fig. 1A). Mature specimens, paired and unpaired, were situated next to a lunule on the oral side of the burrowing sand dollar *Astriclypeus manni,* which is considered to be their host (Fig. 1B, C). The female reaches upward to hook the terminal half of two arms over the edge of the lunule. She firmly fixes her aboral surface to the oral surface of the sand dollar by attaching her aboral skeletal elements to the oral spines of the host. Two of the tips of the male's arms are just visible protruding from under the female's disk (Fig. 1D). Most ophiuroids, whether paired or unpaired, were located at the lunule of the sand dollar as an attachment site, from which a radial food track leads away food particles to the sand dollar's mouth. However, some single young females and males were not located at the lunule, but on the oral plate of the sand dollar, closer to its mouth and anus.

In addition to the size dimorphism, external morphological differences between males and females are evident in such skeletal characteristics as the shape of the jaw, the number and size of disk scales, the number of arm spines, the presence or absence of parallel grooves on the radial shields and disk scales of the aboral disk, and tentacle scales on the oral side of the arm. The jaws of both males and females, present at each interradius, consist of one tooth, one oral shield, two adoral shields, two oral plates with infradental papillae, and two buccal scales. The jaw ossicles of females are stouter than those of males, and the oral plates and teeth are more apparent in the female (Fig. 2A, B). The aboral side of the smaller disk of the male is covered with scales that are less reduced in size and number than those of the female (Fig. 2C). The lateral arm plates of

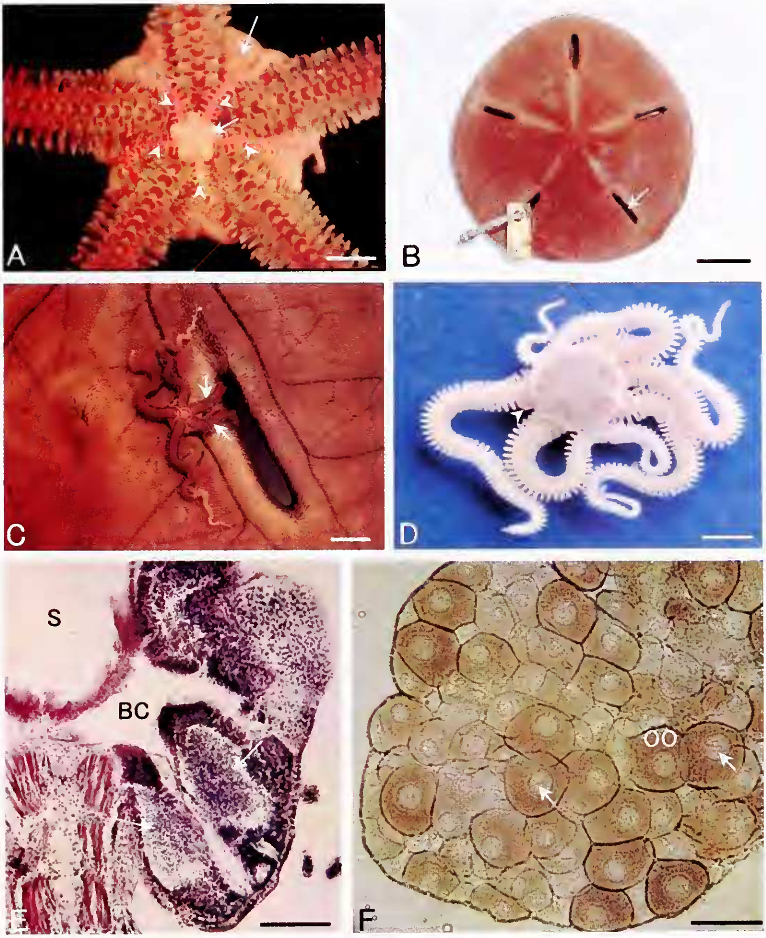

Figure 1. Male and female of *Ophiodaphne formata* and their host, *Astriclypeus manni.* (A) Magnified view of the female paired with a much smaller male of *O. formata,* both detached from the host shown in C. Note the dwarf male (short arrow), with his oral surface against that of the larger female (long arrow), and his arms (arrowheads) alternating with hers. Views of the female and male are oral and aboral, respectively. (B) Aboral view of a sand dollar, *A. manni.* Arrow indicates a lunule. (C) Female specimen of *O. formata* carrying a dwarf male close to the lunule on the oral side of *A. manni.* Note her position, with two arms (arrows) hooked over the edge of the lunule. (D) Aboral view of the female. The body of the male is hidden by the female, but the tips of two of his arms (arrowheads) are visible. (E) Horizontal section of a male specimen of *O. formata* with mature sperm (arrows) in a pair of testes situated at the interradius. The space above the testes is a body cavity (BC) near the stomach (S). (F) Compressed ovary filled with ova (OO) with germinal vesicle (arrows). Scale bars: 3 cm (B), 3 mm (C, D), 1 mm (A), and 100 μm (E, F).

males have 4 spines, whereas those of females have 8. Females possess grooves on their radial shields and disk scales of the disk (Fig. 2D, E) and tentacle scales on the oral side of the arm, while males do not (Fig. 2F, G).

In contrast to the sexual dimorphism in adults, recently metamorphosed juveniles, whose disks are about 400 μm in diameter, do not vary morphologically among individuals. However, the smallest specimen collected on a sand dollar

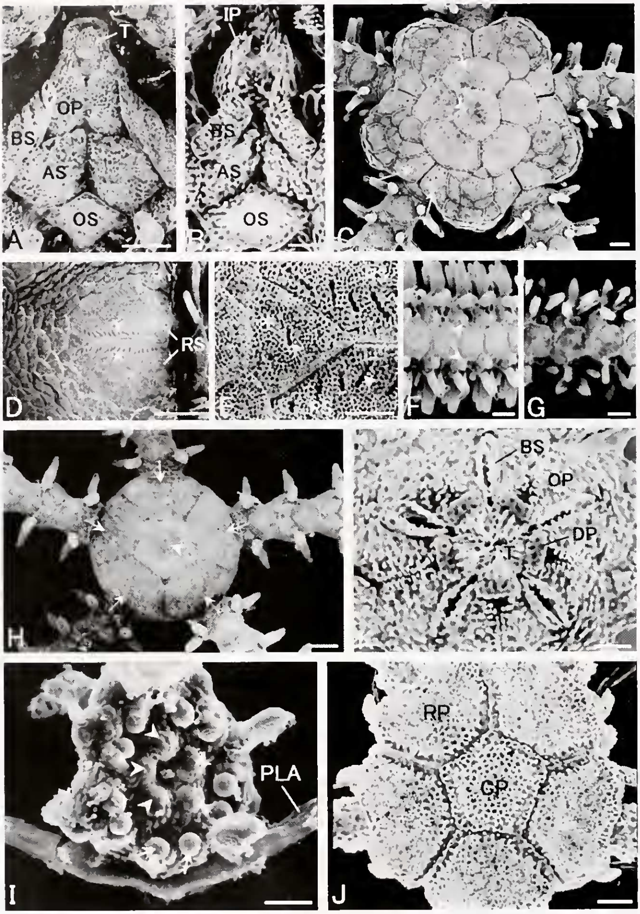

Figure 2. Skeletal structures of *Ophiodaphne formata.* A–K are scanning electron micrographs. (A) Adult female jaw in an interradius; oral view. Components: an oral shield (OS), two adoral shields (AS), two oral plates (OP), and a tooth (T). BS, buccal scale. (B) Adult male jaw in an interradius; oral view. Components of the jaw are the same as in female, but compare structures. IP, infradental papilla; other abbreviations as in (A). (C) Aboral skeletal system of an adult male detached from the host. Note that the disk is covered with scales: a central plate (arrowhead), five radial plates (short arrows), five pairs of radial shields (long arrows), and others. (D) Aboral view of grooves (arrowheads) on the radial shields (RS) of a female. (E) Grooves on the radial shields (RS) and the disk scale (asterisk) of a female at high magnification. (F) Oral view of tentacle scales (arrowheads) on the arm of a female. (G) Oral view of the arm of a male. Note absence of tentacle scales. (H) Aboral skeletal system of a young male brittle star detached from the host. Note the central plate (arrowhead) and five radial plates (arrows) on his disk. (I) Oral view of metamorphosing ophiopluteus with juvenile mouth formation and postero-lateral arm (PLA). Oral tube feet (arrowheads) are visible around the mouth, and tube feet (arrows) are more distal. (J) Central plate (CP) and five radial plates (RP) of a newly metamorphosed juvenile. Aboral view. (K) Oral view of jaws consisting of oral plates (OP), dental plates (DP), and tooth (T). BS indicates buccal scale. Same stage as in J. Scale bars: 500 μm (D, F), 200 μm (G), 100 μm (A, C, E, H), and 30 μm (B, I–K).

from the field (disk diameter 480 μm) exhibited male characteristics in the ossicle of the jaw and skeletal elements of the disk (Fig. 2H). The disk diameter of the smallest female specimen collected from the field was already 1.0 mm. These observations suggest that size differences corresponding to sexual dimorphism first appear in individuals with disk diameters of about 500 μm (males) and 1 mm (females).

Gonadal development and spawning

Sex in *O. formata* is distinguished by the color of the gonads upon dissection; the testes are creamy white, and the ovaries are pale pink. The gonads of both males and females were largest in specimens collected in August. Sections of gonad show that the testes are occupied, in early August, with numerous mature sperm (Fig. 1E), while the ovaries contain numerous oocytes, many of them fully grown and with a germinal vesicle (Fig. 1F). Later in August, after spawning, the ovaries are still large, but they contain no fully grown oocytes, and the center of the organ is occupied by a wide cavity, indicating degeneration. In October and December, the ovaries are smaller than in August. Though ovaries examined from January to May remain small, they are filled with developing oogonia and a few oocytes. Spawning in the laboratory begins when a paired female raises her disk from the bottom of the glass vessel to assume a shedding posture. The eggs and sperm are shed into each bursa, and are released outside through the genital slits at the base of the arms. The release is immediately followed by external fertilization.

We did not attempt to observe spawning in the field. However, our histological study of gonadal development—and our observation that eggs fertilized in August (but not in June, July or October) completed metamorphosis and developed into juveniles—all suggest that, in Tsuruga Bay, the breeding season for *O. formata* occurs during August.

Development

Early development. Fertilized eggs are spherical, about 90 μm in diameter, pale pink, and negatively buoyant (Fig. 3A). They have a transparent, nonsticky fertilization envelope, and a translucent, thick (10 μm) hyaline layer. A chronology of development, from fertilized egg to juvenile, is presented in Table 1. The cleavage is total, equal, and radial. At about 26 °C, the first division occurs at 2 h after fertilization, and as divisions continue (Fig. 3B), the embryos develop into blastulae (Fig. 3C). These blastulae are not wrinkled, unlike those of two other ophiuroids (*Ophiothrix oerstedi* and *Ophionereis schayeri*) and members of other echinoderm classes (Mladenov, 1979; Henry *et al.*, 1991; Chia *et al.*, 1993; Selvakumaraswamy and Byrne, 2000; Komatsu *et al.*, 2000). Nine hours after fertilization, the blastula hatches from the fertilization envelope (Fig. 3D), and primary mesenchyme cells in the vegetal pole wall are set free into the blastocoel (Fig. 3E). At this stage, blastulae in culture swim actively just beneath the water's surface. They become oval (180 μm long and 120 μm wide); and 12 h after fertilization, gastrulation occurs by invagination at the vegetal pole. During gastrulation, the embryo flattens dorso-ventrally (Fig. 3F).

Ophiopluteus stage. Twenty hours after fertilization, in the gastrula stage, the larval spicules begin to take a tetraradiate form (Fig. 3G, H). Then a pair of right and left coelomic pouches is formed on both sides of the tip of the archenteron. Figure 3I shows an early 2-armed ophiopluteus, 35 h after fertilization, taking the shape of a helmet as the postero-lateral arms appear. The antero-lateral arm buds are evident 60 h after fertilization (Fig. 3J). In this early 4-armed ophiopluteus, the archenteron has differentiated into a functional digestive tract: esophagus and stomach (Fig. 3J).

From the 2-armed to the 4-armed stage, two pair of recurrent rods arise successively, running parallel to the body rods (Fig. 3K). These recurrent rods extend to the center of the larval basket-like structure from paired points of divergence, and the body rods also arise from these points (DP in Fig. 3K). Thus, these recurrent rods, together with the body rods and transverse rods, constitute a bilateral, threefold skeletal structure. Immediately after the postero-lateral, antero-lateral, and body rods form, the post-oral rods also appear, and the postero-lateral rods extend horizontally and support the postero-lateral arms.

Four and a half days after fertilization, the post-oral arms are formed, and the ophioplutei develop to the 6-armed stage (Fig. 4A). Six and a half days after fertilization, they become 8-armed ophioplutei, bearing a 4th pair of arms, the postero-dorsal arms (Fig. 4C). At this stage, both the right and left coelomic pouches, the latter of which is further developed than the former, are divided into anterior and posterior sections on each side (Fig. 3L).

The body, the postero-lateral, the antero-lateral, the post-oral, and the postero-dorsal rods are not fenestrated and have no thorns. The length of the postero-lateral arm in the largest 8-armed ophiopluteus larvae is about 700 μm, and the postero-lateral rod is a spiral structure in the middle of this arm (Fig. 4C). These larval arm rods serve as flotation devices, and are absorbed as metamorphosis proceeds. Late 8-armed ophioplutei have neither ciliary epaulets nor vibratile lobes. The left posterior coelomic pouch is divided into a hydrocoel and somatocoel, and the former expands forward, gradually, along the stomach and esophagus, producing a 5-lobed hydrocoel (Fig. 3M, N and 4B). A hydrocoel lobe forms, passes through the left posterior coelomic pouch, and migrates around the esophagus of the 8-armed ophiopluteus larva just before the beginning of metamorphosis. After migrating and surrounding the esophagus for about a day, the 5-lobed hydrocoel develops into the water

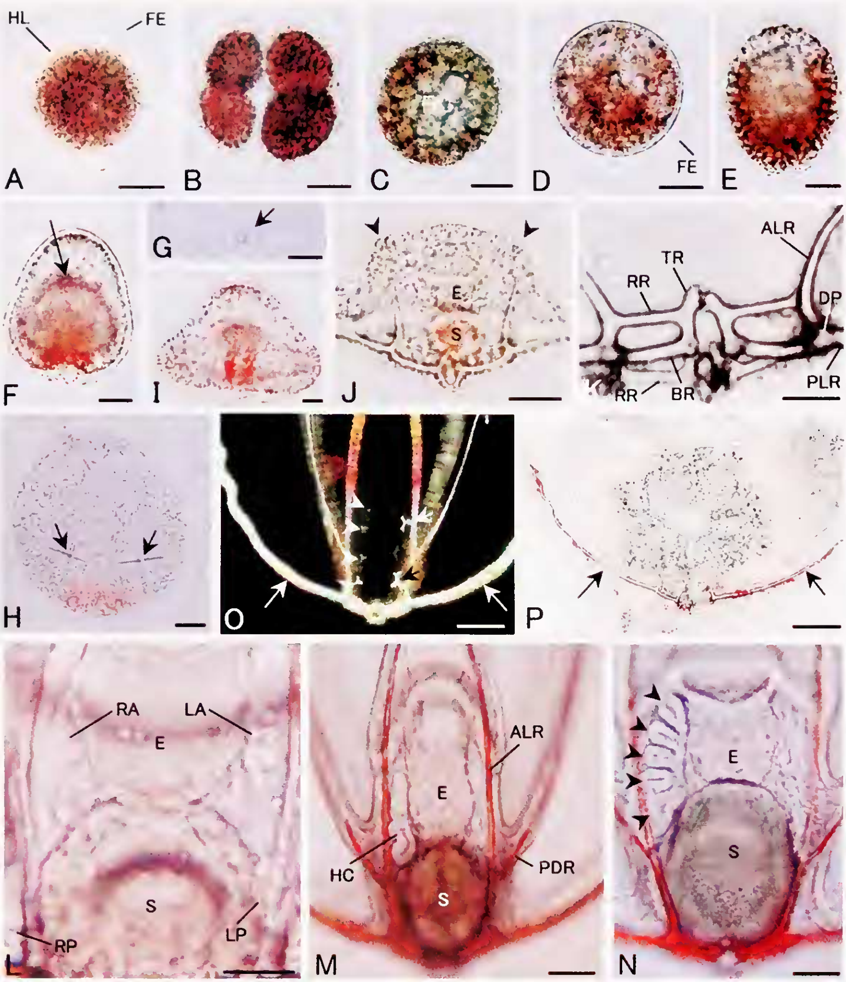

Figure 3. Early development of *Ophiodaphne formata.* (A–N and P are light micrographs; O is a polarized light micrograph.) (A) Fertilized egg surrounded by the fertilization envelope (FE) and hyaline layer (HL). (B) Four-cell stage, 2.5 h after fertilization. (C) Blastula with blastocoel (BC), 6.2 h after fertilization. (D) Hatching blastula, 9 h after fertilization. Note fertilization envelope (FE). (E) Swimming blastula. (F) Gastrula, 21 h after fertilization. Arrow indicates the archenteron. (G) Tetraradiate spicule (arrow) in gastrula at high magnification. (H) A pair of tetraradiate spicules (arrows) in a compressed gastrula; later stage than that shown in G. (I) Early 2-armed ophiopluteus, 1.5 d after fertilization. (J) Early 4-armed ophiopluteus with the antero-lateral arm buds (arrowheads), esophagus (E), and stomach (S), 2.5 d after fertilization. (K) Magnified view of skeletal structure of late 4-armed ophiopluteus. Two pairs of recurrent rods (RR) run parallel to the body rod (BR), and perpendicular to the transverse rods (TR), from the diverging points (DP). The postero-lateral rod (PLR) and antero-lateral rod (ALR) also arise from the diverging points. (L) Magnified view of esophagus (E) and stomach (S) in an 8-armed ophiopluteus. The right and left, anterior (RA, LA) and posterior (RP, LP) coelomic pouches are indicated. Oral view. (M) Late 8-armed ophiopluteus with hydrocoel (HC) along the stomach (S) and esophagus (E). Orange structures are antero-lateral rod (ALR) and postero-dorsal rod (PDR). Aboral view. (N) 8-armed ophiopluteus, more advanced than that shown in (M), with five-lobed hydrocoel (arrowheads) beside

Table 1

Chronology of development of Ophiodaphne formata *(26 °C)*

Time after fertilization	Stage
2 h	2-cell stage
2.5 h	4-cell stage
3 h	8-cell stage
3.5 h	16-cell stage
5 h	Morula
6.2 h	Blastula with blastocoel
9 h	Hatching
21 h	Gastrula, 140 μm long and 120 μm wide
1.5 d	2-armed ophiopluteus
2.5 d	4-armed ophiopluteus
4.5 d	6-armed ophiopluteus
6.5 d	8-armed ophiopluteus with posterior coelom
13.5 d	Hydrocoel formation
15.5 d	Left hydrocoel 5-lobed
18.5 d	Rudiments of adult skeleton appear as spicules
20.5 d	Metamorphic climax begins, larval arm degenerates rapidly
21.5 d	Completion of metamorphosis with absorption of larval arms

vascular system. The 6-armed and 8-armed ophioplutei have a mass of pigment cells including pigment granules, brownish and deep reddish, at the tips of the postero-lateral arms.

Metamorphosis. Eighteen and a half days after fertilization, the adult plates begin to form as fine spicules (Fig. 3O). These spicules correspond to the rudiments of the five terminal plates and large radial plates with a dorso-central plate. At this stage, the stomach and intestine are greenish. In the 8-armed ophiopluteus, 20 days after fertilization, the tip of the postero-lateral arms begin to swell, and the antero-lateral arms cross (Fig. 4C). Absorption of the post-oral and postero-dorsal arms begins 21 days after fertilization (Fig. 4D). Absorption of the left antero-lateral arm occurs and is followed by a decrease in the size of the right antero-lateral arm. Toward the end of metamorphosis, the left antero-lateral arm becomes much shorter than the right (Fig. 4E). The disk consists of small spicules that develop as a skeletal network (Fig. 3P). The spicules that will differentiate to become terminal and radial plates will migrate and will be situated at the tip of the arm and the margin of the disk. The metamorphosing larva is furnished by the rudimentary ophiuroid with a mouth, paired oral tube feet and tube feet; and it frequently creeps along the bottom (Fig. 2I).

Twenty-one and a half days after fertilization, metamorphosis is complete, and the left postero-lateral arm is absorbed, followed by the right (Table 1, Fig. 4F). Immediately after metamorphosis, the juveniles are pentagonal with short arms and spines (Fig. 4G). These paired short spines between the arms disappear as the juveniles grow (Fig. 1D, 2C). Newly metamorphosed juveniles are about 270 μm in disk diameter. They bear a terminal tentacle in the tip of each arm. On their aboral side, a central plate is situated in the center of the disk, surrounded by five radial plates (Fig. 2J). On the oral side, rudiments of five jaws begin to form (Fig. 2K). At this stage, the external morphology of skeletal elements does not vary among specimens.

After a period of 45 days (post-fertilization), the juveniles grow to approximately 400 μm in disk diameter, and are brown. They have five arms, each 130 μm long, consisting of one segment and a terminal plate (Fig. 4H). Although more than 200 juveniles survived in the laboratory for about 2 months after fertilization, they did not differentiate further and eventually died. One specimen collected from the natural habitat on 14 January 2002 was 480 μm in disk diameter (Fig. 2H). It possessed about 11 segments in each of its arms, which were approximately 1.6 mm long. We estimate that this field specimen was about 5 months old.

Discussion

We have described here, for the first time, the development—from spawning and fertilization through metamorphosis—of the sexually dimorphic ophiuroid *Ophiodaphne formata.* The pattern of development in *O. formata* is influenced by four characteristics. The egg is small, which is consistent with the observed indirect development through a planktotrophic ophiopluteus. However, the formation of tetraradiate larval spicules and the absence of a secondary vitellaria larva are features that tend to reduce the time to metamorphosis. Finally, the ciliated postero-lateral arms are retained, which may provide the juvenile brittle star with mobility for a brief presettlement exploration of the substrate. This suite of developmental characteristics is in accord with the novel natural history of *O. formata,* in which a dwarf male and a female are coupled and attached to the oral surface of the sand dollar *Astriclypeus manni,* mostly adjacent to the lunule.

Methods of inducing spawning in ophiuroids—except for a sudden change in water temperature reported for *Amphipholis kochii* by Yamashita (1985)—are not as precise or

esophagus (E) and upper part of stomach (S), 15.5 d after fertilization. Aboral view. (O) Skeletal system of late 8-armed ophiopluteus (polarized light micrograph). Postero-lateral rods (long arrows); rudimental radial plates (short arrows); terminal plates (arrowheads). Aboral view. (P) A pair of postero-lateral rods (arrows) of a metamorphosing ophiopluteus, compressed. Note the skeletal network for the resulting juvenile. Oral view. Scale bars: 100 μm (N, O, P), 50 μm (J, L), and 30 μm (A–I, K, M).

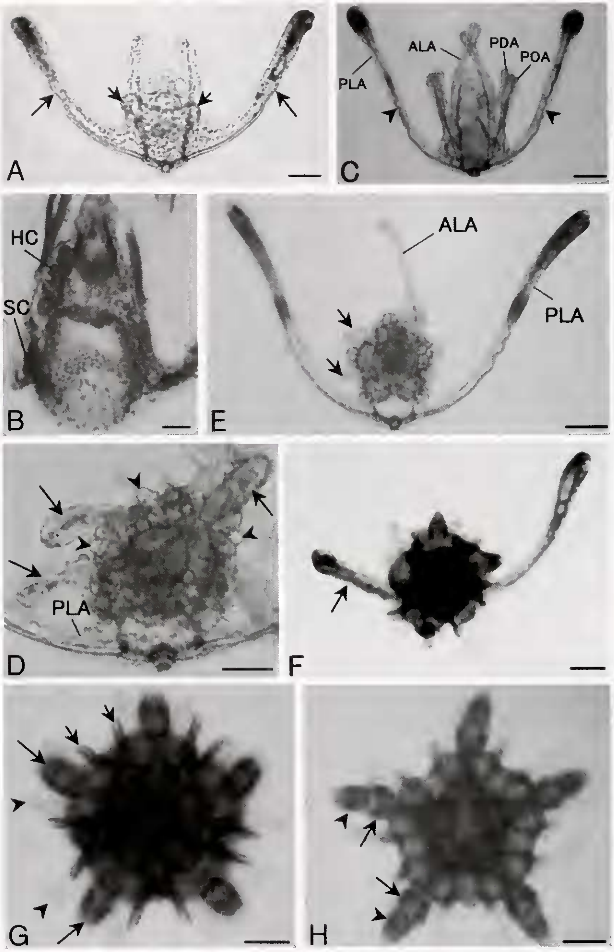

Figure 4. Development of *Ophiodaphne formata.* (A) Early 6-armed ophiopluteus, 4.5 d after fertilization. Long arrows indicate the postero-lateral arms, and short arrows, the post-oral arms. Oral view. (B) Histological longitudinal section (4 μm) of an 8-armed ophiopluteus showing the hydrocoel (HC) and somatocoel (SC). Same stage as shown in Figure 3N. Aboral view. (C) Metamorphosing ophiopluteus; oral view. Note the swollen tip of the postero-lateral arms (PLA), the crossed antero-lateral arms (ALA), and the spiral construction of the postero-lateral rods (arrowheads). (D–F) Successive stages of resorption of the larval arms. (D) Magnified aboral view of the ophiuroid rudiment, showing tube feet (arrowheads). The rudiment is within the metamorphosing ophiopluteus, which has a pair of postero-lateral arms (PLA) and reduced post-oral and postero-dorsal arms (arrows). (E) Metamorphosing ophiopluteus with a right antero-lateral arm (ALA) and other reduced larval arms (arrows) hanging on postero-lateral arms (PLA). Aboral view of the rudiment. (F) Metamorphosing ophiopluteus with a shorter left postero-lateral arm (arrow) than right, 20.5 d after fertilization. Aboral view. (G) Juvenile just after metamorphosis with terminal plates (long arrows), spines (short arrows), and tube feet (arrowheads), 21.5 d after fertilization. Aboral view. (H) Juvenile with arm segments (arrows). Arrowheads indicate terminal plates. Aboral view. Scale bars: 100 μm (C, E, F, H), and 50 μm (A, B, D, G).

reliable as those known for echinoids or asteroids (Strathmann and Rumrill, 1987). Fortunately, however, *O. formata* spawns spontaneously in the laboratory, so the entire process of development, from fertilized eggs to juveniles, has been observed in this study. The entire process has been observed in several other species: *Ophiothrix fragilis*, *Ophiocoma nigra*, *Ophiopholis aculeata*, *Ophiocoma pumila*, and *A. kochii* (MacBride, 1907; Narasimhamurti, 1933; Olsen, 1942; Mladenov, 1985; Yamashita, 1985), but none of these is a sexually dimorphic species. This study, therefore, is the first demonstration of a sexually dimorphic ophiuroid, developing through a typical ophiopluteus stage, and then into an 8-armed planktotrophic larva.

The mature ova of *O. formata* are 90 μm in diameter, similar in size to those of *O. fragilis*, *O. nigra*, and *A. kochii* (MacBride, 1907; Narasimhamurti, 1933; Yamashita, 1985). Mladenov (1979) summarized the quantitative characteristics of developmental patterns in ophiuroids and noted that species with small eggs (70–200 μm in diameter) undergo planktotrophic development and require 12–40 days to reach metamorphic competence. In the present study, *O. formata* completed metamorphosis within 21.5 days at 26 °C and thus fits the categorization of Mladenov (1979), as do *O. fragilis*, *O. nigra*, and *A. kochii*. Of these species, *O. fragilis* and *O. nigra* occur in relatively deep waters, whereas *A. kochii* is found under stones in the intertidal zone, along the Pacific coast of northern Japan, and *O. formata* inhabits the sandy bottom, 5 m deep, at Tsuruga Bay. A rapid metamorphosis should be advantageous to the shallow-water brittle stars, *O. formata* and *A. kochii*, for it would prevent dispersal to less advantageous deep habitats. In the case of the reproductive pairs of *O. formata*, which always live on a host sand dollar on the shallow sandy bottom, the requirement for rapid metamorphosis may be especially important.

Hyman (1955) generalized that an early ophiopluteus is furnished with a three-rayed skeletal rod, and Olsen (1942) and Strathmann and Rumrill (1987) reported this condition in *Amphipholis squamata* and *O. aculeata*, respectively. However, the present study reveals that the initial shape of the larval spicules in *O. formata* is tetraradiate, as in *Amphiura chiajei*, *Amphioplus abditus*, *A. kochii*, and *O. schayeri* (Fenaux, 1963; Hendler, 1978; Yamashita, 1985; Selvakumaraswamy and Byrne, 2000). Therefore, the rudiments of the skeletal rods in the ophiopluteus can form in two ways: triradiate or tetraradiate. The accelerated formation of tetraradiate spicules in the gastrula stage may reduce the time to metamorphosis and thus contribute to the rapid embryonic development in *O. formata*.

At settlement, ophioplutei generally release their postero-lateral arms (Olsen, 1942; Byrne and Selvakumaraswamy, 2002). In *O. formata*, however, the four pairs of larval arms are not discarded, but rather absorbed—first the post-oral and postero-dorsal arms, and then the antero-lateral and postero-lateral arms. Thus, the report of Balser (1998), that the released arms of an ophiopluteus can regenerate all the structures typical of the primary ophiopluteus, and that asexual reproduction of larval arms may be highly adaptive for life in the open ocean, does not apply to *O. formata*.

Ophiuroids live in all seas, in all types of sediment, and at all depths—from the intertidal zone to the abyssal region. Among these species, only *O. formata* is found on sand dollars, such as *A. manni* (Tominaga, 2001, and present materials), *Clypeaster reticulatus* (Irimura, 1981), and *C. japonicus* (Tominaga, unpubl.); and these host organisms are always in shallow waters, partially buried in the sandy bottom. In this study, the ophiuroids were never found on the sandy bottom; rather, the much larger female, carrying a dwarf male in a mouth-to-mouth position, is herself attached, by her aboral side, to the oral surface of the host *A. manni*. The lunule of the host may serve the female ophiuroid as a convenient site for attachment, or the concave shape of the lunule may provide protection from abrasion by the sand. Although we might suggest that a radial food track of the sand dollar, located close to the edge of the lunule (Fig. 1C), provides nutriment for the paired ophiuroids, this seems unlikely, because paired and unpaired females on the oral side of the sand dollar always turn their mouths to the sandy bottom to feed, not to a radial food track. Probably the association between the male and female and their morphological specializations have evolved as an adaptation to ensure mating success on this mobile and infaunal host. Although the pairing in *O. formata* is observed throughout the year, including in the nonbreeding season, this pairing behavior is probably essential to their reproduction, because spawning occurs while pairing.

Males and females of *O. formata* have a bursa on the oral surface which provides an opening for the gonad. Consequently, the most efficient posture for the male is to interdigitate his arms with the larger female, mouth to mouth, while he sheds sperm from his bursal slits. The posture is important because the low density of *O. formata* is in contrast to that of the more common shallow-water ophiuroids (Fujita, 1992). Thus, fertilization efficiency would probably be low if males and females of *O. formata* spawned separately on the sand and did not pair on their host. Probably *O. formata* selects *A. manni* as a host that provides a breeding site and thus raises the level of fertilization success, as suggested by Hendler (1991).

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